(39)

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/53, 15/82, A01H 5/00 | A2

(11) International Publication Number: WO 96/21022

(43) International Publication Date: 11 July 1996 (11.07.96)

(21) International Application Number: PCT/IB95/01167

(22) International Filing Date: 28 December 1995 (28.12.95)

(30) Priority Data:
08/366,779 30 December 1994 (30.12.94) US

(71) Applicant: RHONE-POULENC AGROCHIMIE [FR/FR]; 14-20, rue Pierre-Baizet, F-69263 Lyon (FR).

(72) Inventors: THOMAS, Terry, L.; 3004 Normand, College Station, TX 77845 (US). REDDY, Avutu, S.; 3902 E. 29th Street #G11, Bryan, TX 77802 (US). NUCCIO, Michael; P.O. Box 553, College Station, TX 77841 (US). NUNBERG, Andrew, N.; 2804 B. Sprucewood Street, Bryan, TX 77801 (US). FREYSSINET, Georges, L.; 21, rue de Nervieux, F-69450 Saint-Cyr-au-Mont-d'Or (FR).

(74) Agent: MITSCHERLICH & PARTNER; Sonnenstrasse 33, D-80331 München (DE).

(81) Designated States: AU, BR, CA, CN, JP, RO, RU, UA, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: PRODUCTION OF GAMMA LINOLENIC ACID BY A Δ6-DESATURASE

(57) Abstract

Linoleic acid is converted into γ-linolenic acid by the enzyme Δ6-desaturase. The present invention is directed to isolated nucleic acids comprising the Δ6-desaturase gene. More particularly, the isolated nucleic acid comprises the promoter, coding region and termination regions of the Δ6-desaturase gene. The present invention provides recombinant constructions comprising the Δ6-desaturase coding region in functional combination with heterologous regulatory sequences. The nucleic acids and recombinant constructions of the instant invention are useful in the production of GLA in transgenic organisms.

PRODUCTION OF GAMMA LINOLENIC ACID BY A Δ6-DESATURASE

Linoleic acid (18:2) (LA) is transformed into gamma linolenic acid (18:3) (GLA) by the enzyme Δ6-desaturase. When this enzyme, or the nucleic acid encoding it, is transferred into LA-producing cells, GLA is produced. The present invention provides nucleic acids comprising the Δ6-desaturase gene. More specifically, the nucleic acids comprise the promoters, coding regions and termination regions of the Δ6-desaturase genes. The present invention is further directed to recombinant constructions comprising a Δ6-desaturase coding region in functional combination with heterologous regulatory sequences. The nucleic acids and recombinant constructions of the instant invention are useful in the production of GLA in transgenic organisms.

Unsaturated fatty acids such as linoleic ($C_{18}\Delta^{9,12}$) and α-linolenic ($C_{18}\Delta^{9,12,15}$) acids are essential dietary constituents that cannot be synthesized by vertebrates since vertebrate cells can introduce double bonds at the Δ^9 position of fatty acids but cannot introduce additional double bonds between the Δ^9 double bond and the methyl-terminus of the fatty acid chain. Because they are precursors of other products, linoleic and α-linolenic acids are essential fatty acids, and are usually obtained from plant sources. Linoleic acid can be converted by mammals into γ-linolenic acid (GLA, $C_{18}\Delta^{6,9,12}$) which can in turn be converted to arachidonic acid (20:4), a critically

allowing production of large amounts of GLA, the present invention provides new dietary sources of GLA.

The present invention is directed to isolated Δ6-desaturase genes. Specifically, the isolated genes comprises the Δ6-desaturase promoters, coding regions, and termination regions.

The present invention is further directed to expression vectors comprising the Δ6-desaturase promoter, coding region and termination region.

Yet another aspect of this invention is directed to expression vectors comprising a Δ6-desaturase coding region in functional combination with heterologous regulatory regions, i.e. elements not derived from the Δ6-desaturase gene.

Cells and organisms comprising the vectors of the present invention, and progeny of such organisms, are also provided by the present invention.

A further aspect of the present invention provides isolated bacterial Δ6-desaturase. An isolated plant Δ6-desaturase is also provided.

Yet another aspect of this invention provides a method for producing plants with increased gamma linolenic acid content.

A method for producing chilling tolerant plants is also provided by the present invention.

Fig. 1 depicts the hydropathy profiles of the deduced amino acid sequences of Synechocystis Δ6-desaturase (Panel A) and Δ12-desaturase (Panel B). Putative membrane spanning regions are indicated by solid bars. Hydrophobic index was calculated for a

of the plasmid is pBI221 and in 121.Δ6.NOS, the remaining portion of the plasmid is pBI121.

Fig. 8 provides gas liquid chromatography profiles of mock transfected (Panel A) and 221.Δ6.NOS transfected (Panel B) carrot cells. The positions of 18:2, 18:3 α, and 18:3 γ(GLA) are indicated.

Fig. 9 provides gas liquid chromatography profiles of an untransformed tobacco leaf (Panel A) and a tobacco leaf transformed with 121.Δ6.NOS. The positions of 18:2, 18:3 α, 18:3γ(GLA), and 18:4 are indicated.

Fig. 10 provides gas liquid chromotography profiles for untransformed tobacco seeds (Panel A) and seeds of tobacco transformed with 121.Δ6.NOS. The positions of 18:2, 18:3α and 18:3γ(GLA) are indicated.

The present invention provides isolated nucleic acids encoding Δ6-desaturase. To identify a nucleic acid encoding Δ6-desaturase, DNA is isolated from an organism which produces GLA. Said organism can be, for example, an animal cell, certain fungi (e.g. Mortierella), certain bacteria (e.g. Synechocystis) or certain plants (borage, Oenothera, currants). The isolation of genomic DNA can be accomplished by a variety of methods well-known to one of ordinary skill in the art, as exemplified by Sambrook et al. (1989) in Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, NY. The isolated DNA is fragmented by physical methods or enzymatic digestion and cloned into an appropriate vector, e.g. a bacteriophage or cosmid vector, by any of a variety of well-known methods which can be found

Anabaena strain PCC 7120, ATCC 27893. Production of GLA from Anabaena linoleic acid is monitored by gas chromatography and the corresponding DNA fragment is isolated.

The isolated DNA is sequenced by methods well-known to one of ordinary skill in the art as found, for example, in Sambrook et al. (1989).

In accordance with the present invention, DNA molecules comprising Δ6-desaturase genes have been isolated. More particularly, a 3.588 kilobase (kb) DNA comprising a Δ6-desaturase gene has been isolated from the cyanobacteria Synechocystis. The nucleotide sequence of the 3.588 kb DNA was determined and is shown in SEQ ID NO:1. Open reading frames defining potential coding regions are present from nucleotide 317 to 1507 and from nucleotide 2002 to 3081. To define the nucleotides responsible for encoding Δ6-desaturase, the 3.588 kb fragment that confers Δ6-desaturase activity is cleaved into two subfragments, each of which contains only one open reading frame. Fragment ORF1 contains nucleotides 1 through 1704, while fragment ORF2 contains nucleotides 1705 through 3588. Each fragment is subcloned in both forward and reverse orientations into a conjugal expression vector (AM542, Wolk et al. [1984] Proc. Natl. Acad. Sci. USA 81, 1561) that contains a cyanobacterial carboxylase promoter. The resulting constructs (i.e. ORF1(F), ORF1(R), ORF2(F) and ORF2(R)] are conjugated to wild-type Anabaena PCC 7120 by standard methods (see, for example, Wolk et al. (1984) Proc. Natl. Acad. Sci. USA 81, 1561). Conjugated cells of Anabaena are

Table 1: Occurrence of C18 fatty acids in wild-type and transgenic cyanobacteria

SOURCE	18:0	18:1	18:2	γ18:3	α18:3	18:4
Anabaena (wild type)	+	+	+	-	+	-
Anabaena + ORF1(F)	+	+	+	-	+	-
Anabaena + ORF1(R)	+	+	+	-	+	-
Anabaena + ORF2(F)	+	+	+	+	+	+
Anabaena + ORF2(R)	+	+	+	-	+	-
Synechocystis (wild type)	+	+	+	+	-	-

As assessed by GLC analysis, GLA deficient Anabaena gain the function of GLA production when the construct containing ORF2 in forward orientation is introduced by transconjugation. Transconjugants containing constructs with ORF2 in reverse orientation to the carboxylase promoter, or ORF1 in either orientation, show no GLA production. This analysis demonstrates that the single open reading frame (ORF2) within the 1884 bp fragment encodes Δ6-desaturase. The 1884 bp fragment is shown as SEQ ID NO:3. This is substantiated by the overall similarity of the hydropathy profiles between Δ6-desaturase and Δ12-desaturase [Wada et al. (1990) Nature 347] as shown in Fig. 1 as (A) and (B), respectively.

Also in accordance with the present invention, a cDNA comprising a Δ6-desaturase gene from borage (Borago officinalis) has been isolated. The nucleotide sequence of the 1.685 kilobase (kb) cDNA

liquid cultures and sequenced. For example, as a means of eliminating other seed storage protein cDNAs from a cDNA library made from borage polysomal RNA, cDNAs corresponding to abundantly expressed seed storage proteins are first hybridized to the cDNA library. The "subtracted" DNA library is then used to generate expressed sequence tags (ETSs) and such tags are used to scan a data base such as GenBank to identify potential desaturates.

Transgenic organisms which gain the function of GLA production by introduction of DNA encoding Δ-desaturase also gain the function of octadecatetraeonic acid ($18:4^{\Delta 6,9,12,15}$) production. Octadecatetraeonic acid is present normally in fish oils and in some plant species of the Boraginaceae family (Craig et al. [1964] J. Amer. Oil Chem. Soc. 41, 209-211; Gross et al. [1976] Can. J. Plant Sci. 56, 659-664). In the transgenic organisms of the present invention, octadecatetraenoic acid results from further desaturation of α-linolenic acid by Δ6-desaturase or desaturation of GLA by Δ15-desaturase.

The 359 amino acids encoded by ORF2, i.e. the open reading frame encoding Synechocystis Δ6-desaturase, are shown as SEQ. ID NO:2. The open reading frame encoding the borage Δ6-desaturase is shown in SEQ ID NO: 5. The present invention further contemplates other nucleotide sequences which encode the amino acids of SEQ ID NO:2 and SEQ ID NO: 5. It is within the ken of the ordinarily skilled artisan to identify such sequences which result, for example, from the degeneracy of the genetic code. Furthermore,

DNA or RNA molecules engineered for controlled expression of a desired gene, i.e. the Δ6-desaturase gene. Preferably the vectors are plasmids, bacteriophages, cosmids or viruses. Shuttle vectors, e.g. as described by Wolk et al. (1984) Proc. Natl. Acad. Sci. USA, 1561-1565 and Bustos et al. (1991) J. Bacteriol. 174, 7525-7533, are also contemplated in accordance with the present invention. Sambrook et al. (1989), Goeddel, ed. (1990) Methods in Enzymology 185 Academic Press, and Perbal (1988) A Practical Guide to Molecular Cloning, John Wiley and Sons, Inc., provide detailed reviews of vectors into which a nucleic acid encoding the present Δ6-desaturase can be inserted and expressed. Such vectors also contain nucleic acid sequences which can effect expression of nucleic acids encoding Δ6-desaturase. Sequence elements capable of effecting expression of a gene product include promoters, enhancer elements, upstream activating sequences, transcription termination signals and polyadenylation sites. Both constitutive and tissue specific promoters are contemplated. For transformation of plant cells, the cauliflower mosaic virus (CaMV) 35S promoter and promoters which are regulated during plant seed maturation are of particular interest. All such promoter and transcriptional regulatory elements, singly or in combination, are contemplated for use in the present replicable expression vectors and are known to one of ordinary skill in the art. The CaMV 355 promoter is described, for example, by Restrepo et al. (1990)

as promoter elements to direct the expression of the Δ6-desaturase of the present invention.

Modifications of the nucleotide sequences or regulatory elements disclosed herein which maintain the functions contemplated herein are within the scope of this invention. Such modifications include insertions, substitutions and deletions, and specifically substitutions which reflect the degeneracy of the genetic code.

Standard techniques for the construction of such hybrid vectors are well-known to those of ordinary skill in the art and can be found in references such as Sambrook et al. (1989), or any of the myriad of laboratory manuals on recombinant DNA technology that are widely available. A variety of strategies are available for ligating fragments of DNA, the choice of which depends on the nature of the termini of the DNA fragments. It is further contemplated in accordance with the present invention to include in the hybrid vectors other nucleotide sequence elements which facilitate cloning, expression or processing, for example sequences encoding signal peptides, a sequence encoding KDEL, which is required for retention of proteins in the endoplasmic reticulum or sequences encoding transit peptides which direct Δ6-desaturase to the chloroplast. Such sequences are known to one of ordinary skill in the art. An optimized transit peptide is described, for example, by Van den Broeck et al. (1985) Nature 313, 358. Prokaryotic and eukaryotic signal sequences are

When necessary for the transformation method, the $\Delta 6$-desaturase genes of the present invention can be inserted into a plant transformation vector, e.g. the binary vector described by Bevan (1984) Nucleic Acids Res. 12, 8111. Plant transformation vectors can be derived by modifying the natural gene transfer system of Agrobacterium tumefaciens. The natural system comprises large Ti (tumor-inducing)-plasmids containing a large segment, known as T-DNA, which is transferred to transformed plants. Another segment of the Ti plasmid, the vir region, is responsible for T-DNA transfer. The T-DNA region is bordered by terminal repeats. In the modified binary vectors the tumor-inducing genes have been deleted and the functions of the vir region are utilized to transfer foreign DNA bordered by the T-DNA border sequences. The T-region also contains a selectable marker for antibiotic resistance, and a multiple cloning site for inserting sequences for transfer. Such engineered strains are known as "disarmed" A. tumefaciens strains, and allow the efficient transformation of sequences bordered by the T-region into the nuclear genomes of plants.

Surface-sterilized leaf disks are inoculated with the "disarmed" foreign DNA-containing A. tumefaciens, cultured for two days, and then transferred to antibiotic-containing medium. Transformed shoots are selected after rooting in medium containing the appropriate antibiotic, transferred to soil and regenerated.

encoding Δ6-desaturase into an organism which lacks or has low levels of GLA, but contains LA. In another embodiment, the method comprises introducing one or more expression vectors which comprise DNA encoding Δ12-desaturase and Δ6-desaturase into organisms which are deficient in both GLA and LA. Accordingly, organisms deficient in both LA and GLA are induced to produce LA by the expression of Δ12-desaturase, and GLA is then generated due to the expression of Δ6-desaturase. Expression vectors comprising DNA encoding Δ12-desaturase, or Δ12-desaturase and Δ6-desaturase, can be constructed by methods of recombinant technology known to one of ordinary skill in the art (Sambrook et al., 1989) and the published sequence of Δ12-desaturase (Wada et al [1990] Nature (London) 347, 200-203. In addition, it has been discovered in accordance with the present invention that nucleotides 2002-3081 of SEQ. ID NO:1 encode cyanobacterial Δ12-desaturase. Accordingly, this sequence can be used to construct the subject expression vectors. In particular, commercially grown crop plants are contemplated as the transgenic organism, including, but not limited to, sunflower, soybean, oil seed rape, maize, peanut and tobacco.

The present invention is further directed to a method of inducing chilling tolerance in plants. Chilling sensitivity may be due to phase transition of lipids in cell membranes. Phase transition temperature depends upon the degree of unsaturation of fatty acids in membrane lipids, and thus increasing the degree of unsaturation, for example by introducing

EXAMPLE 1

Strains and Culture Conditions

Synechocystis (PCC 6803, ATCC 27184), Anabaena (PCC 7120, ATCC 27893) and Synechococcus (PCC 7942, ATCC 33912) were grown photoautotrophically at 30°C in BG11N+ medium (Rippka et al. [1979] J. Gen. Microbiol. 111, 1-61) under illumination of incandescent lamps ($60\mu E.m^{-2}.S^{-1}$). Cosmids and plasmids were selected and propagated in Escherichia coli strain DH5α on LB medium supplemented with antibiotics at standard concentrations as described by Maniatis et al. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring, New York.

EXAMPLE 3

Gain-of-Function Expression of GLA in Anabaena

Anabaena (PCC 7120), a filamentous cyanobacterium, is deficient in GLA but contains significant amounts of linoleic acid, the precursor for GLA (Figure 2; Table 2). The Synechocystis cosmid library described in Example 2 was conjugated into Anabaena (PCC 7120) to identify transconjugants that produce GLA. Anabaena cells were grown to mid-log phase in BG11N+ liquid medium and resuspended in the same medium to a final concentration of approximately $2x10^8$ cells per ml. A mid-log phase culture of E. coli RP4 (Burkardt et al. [1979] J. Gen. Microbiol. 114, 341-348) grown in LB containing ampicillin was washed and resuspended in fresh LB medium. Anabaena and RP4 were then mixed and spread evenly on BG11N+ plates containing 5% LB. The cosmid genomic library was replica plated onto LB plates containing 50 μg/ml kanamycin and 17.5 μg/ml chloramphenicol and was subsequently patched onto BG11N+ plates containing Anabaena and RP4. After 24 hours of incubation at 30°C, 30 μg/ml of neomycin was underlaid; and incubation at 30°C was continued until transconjugants appeared.

Individual transconjugants were isolated after conjugation and grown in 2 ml BG11N+ liquid medium with 15 μg/ml neomycin. Fatty acid methyl esters were prepared from wild type cultures and cultures containing pools of ten transconjugants as follows. Wild type and transgenic cyanobacterial

in a region approximately 7.5 kb in length. A 3.5 kb NheI fragment of cSy75 was recloned in the vector pDUCA7 and transferred to Anabaena resulting in gain-of-function expression of GLA (Table 2).

Two NheI/Hind III subfragments (1.8 and 1.7 kb) of the 3.5 kb Nhe I fragment of cSy75-3.5 were subcloned into "pBLUESCRIPT" (Stratagene) (Figure 3) for sequencing. Standard molecular biology techniques were performed as described by Maniatis et al. (1982) and Ausubel et al. (1987). Dideoxy sequencing (Sanger et al. [1977] Proc. Natl. Acad. Sci. USA 74, 5463-5467) of pBS1.8 was performed with "SEQUENASE" (United States Biochemical) on both strands by using specific oligonucleotide primers synthesized by the Advanced DNA Technologies Laboratory (Biology Department, Texas A & M University). DNA sequence analysis was done with the GCG (Madison, WI) software as described by Devereux et al. (1984) Nucleic Acids Res. 12, 387-395.

Both NheI/HindIII subfragments were transferred into a conjugal expression vector, AM542, in both forward and reverse orientations with respect to a cyanobacterial carboxylase promoter and were introduced into Anabaena by conjugation. Transconjugants containing the 1.8 kb fragment in the forward orientation (AM542-1.8F) produced significant quantities of GLA and octadecatetraenoic acid (Figure 2; Table 2). Transconjugants containing other constructs, either reverse oriented 1.8 kb fragment or forward and reverse oriented 1.7 kb fragment, did not produce detectable levels of GLA (Table 2).

Table 2 Composition of C18 Fatty Acids in Wild Type and Transgenic Cyanobacteria

Strain	Fatty Acid (%)					
	18:0	18:1	18:2	18.3(α)	18.3(γ)	18.4
Wild Type						
Synechocystis (sp.PCC6803)	13.6	4.5	54.5	-	27.3	-
Anabaena (sp.PCC7120)	2.9	24.8	37.1	35.2	-	-
Synechococcus (sp.PCC7942)	20.6	79.4	-	-	-	-
***Anabaena* Transconjugants**						
cSy75	3.8	24.4	22.3	9.1	27.9	12.5
cSy75-3.5	4.3	27.6	18.1	3.2	40.4	6.4
pAM542 - 1.8F	4.2	13.9	12.1	19.1	25.4	25.4
pAM542 - 1.8R	7.7	23.1	38.4	30.8	-	-
pAM542 - 1.7F	2.8	27.8	36.1	33.3	-	-
pAM542 - 1.7R	2.8	25.4	42.3	29.6	-	-
***Synechococcus* Transformants**						
pAM854	27.8	72.2	-	-	-	-
pAM854 -Δ^{12}	4.0	43.2	46.0	-	-	-
pAM854 -Δ^{6}	18.2	81.8	-	-	-	-
pAM854 -Δ^{6}&Δ^{12}	42.7	25.3	19.5	-	16.5	-

18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid; 18:3(α), linolenic acid; 18:3(γ), γ-linolenic acid; 18:4, octadecatetraenoic acid

Table 2 shows that the principal fatty acids of wild type Synechococcus are stearic acid (18:0) and oleic acid (18:1). Synechococcus transformed with pAM854-Δ12 expressed linoleic acid (18:2) in addition to the principal fatty acids. Transformants with pAM854-Δ6 and Δ12 produced both linoleate and GLA (Table 1). These results indicated that Synechococcus containing both Δ12- and Δ6-desaturase genes has gained the capability of introducing a second double bond at the Δ12 position and a third double bond at the Δ6 position of C18 fatty acids. However, no changes in fatty acid composition was observed in the transformant containing pAM854-Δ6, indicating that in the absence of substrate synthesized by the Δ12 desaturase, the Δ6-desaturase is inactive. This experiment further confirms that the 1.8 kb NheI/HindIII fragment (Figure 3) contains both coding and promoter regions of the Synechocystis Δ6-desaturase gene. Transgenic Synechococcus with altered levels of polyunsaturated fatty acids were similar to wild type in growth rate and morphology.

EXAMPLE 6

Transfer of Cyanobacterial Δ^6-Desaturase into Tobacco

The cyanobacterial Δ^6-desaturase gene was mobilized into a plant expression vector and transferred to tobacco using Agrobacterium mediated gene transfer techniques. To ensure that the transferred desaturase is appropriately expressed in leaves and developing seeds and that the desaturase gene product is targeted to the endoplasmic reticulum or the chloroplast, various expression cassettes with Synechocystis Δ-desaturase open reading frame (ORF) were constructed. Components of these cassettes include: (i) a 35S promoter or seed specific promoter derived from the sunflower helianthinin gene to drive Δ^6-desaturase gene expression in all plant tissues or only in developing seeds respectively, (ii) a putative signal peptide either from carrot extensin gene or sunflower helianthinin gene to target newly synthesized Δ^6-desaturase into the ER, (iii) an ER lumen retention signal sequence (KDEL) at the COOH-terminal of the Δ^6-desaturase ORF, and (iv) an optimized transit peptide to target Δ^6 desaturase into the chloroplast. The 35S promoter is a derivative of pRTL2 described by Restrepo et al. (1990). The optimized transit peptide sequence is described by Van de Broeck et al. (1985). The carrot extensin signal peptide is described by Chen et al (1985) EMBO J. 9, 2145.

Transgenic tobacco plants were produced containing a chimeric cyanobacterial desaturase gene,

EXAMPLE 7

Construction of Borage cDNA library

Membrane bound polysomes were isolated from borage seeds 12 days post pollination (12 DPP) using the protocol established for peas by Larkins and Davies (1975 Plant Phys. 55:749-756). RNA was extracted from the polysomes as described by Mechler (1987 Methods in Enzymology 152:241-248, Academic Press).

Poly-A+ RNA was isolated from the membrane bound polysomal RNA by use of Oligotex-dT beads (Qiagen). Corresponding cDNA was made using Stratagene's ZAP cDNA synthesis kit. The cDNA library was constructed in the lambda ZAP II vector (Stratagene) using the lambda ZAP II vector kit. The primary library was packaged in Gigapack II Gold packaging extract (Stratagene). The library was used to generate expressed sequence tags (ESTs), and sequences corresponding to the tags were used to scan the GenBank database.

EXAMPLE 9

Random sequencing of cDNAs from a borage seed (12 DPP) membrane-bound polysomal library

The borage cDNA library was plated at low density (500 pfu on 150 mm petri dishes). Highly prevalent seed storage protein cDNAs were "subtracted" by screening with the previously identified corresponding cDNAs. Non-hybridizing plaques were excised using Stratagene's excision protocol and reagents. Resulting bacterial colonies were used to inoculate liquid cultures and were either sequenced manually or by an ABI automated sequencer. Each cDNA was sequenced once and a sequence tag generated from 200-300 base pairs. All sequencing was performed by cycle sequencing (Epicentre). Over 300 ESTs were generated. Each sequence tag was compared to GenBank database by BLASTX computer program and a number of lipid metabolism genes, including the Δ6-desaturase were identified.

Database searches with a cDNA clone designated mbp-65 using BLASTX with the GenBank database resulted in a significant match to the Synechocystis Δ6-desaturase. It was determined however, that this clone was not a full length cDNA. A full length cDNA was isolated using mbp-65 to screen the borage membrane-bound polysomal library. The sequence of the isolated cDNA was determined (Fig. 5A, SEQ ID NO:4) and the protein sequence of the open reading frame (Fig. 5B, SEQ ID NO:5) was compared to other known desaturases using Geneworks

Table 3. Comparison of common amino acid motifs in membrane-bound desaturases

Desaturase	Amino Acid Motif		
	Lipid Box	Metal Box 1	Metal Box 2
Borage Δ^4	WIGHDAGH (SEQ. ID. NO: 6)	HNAHH (SEQ. ID. NO: 12)	FQIEHH (SEQ. ID. NO: 20)
Synechocystis Δ^6	NVGHDANH (SEQ. ID. NO: 7)	HNYLHH (SEQ. ID. NO: 13)	HQVTHH (SEQ. ID. NO: 21)
Arab. chloroplast Δ^{15}	VLGHDCGH (SEQ. ID. NO: 8)	HRTHH (SEQ. ID. NO: 14)	HVIHH (SEQ. ID. NO: 22)
Rice Δ^{15}	VLGHDCGH (SEQ. ID. NO: 8)	HRTHH (SEQ. ID. NO: 14)	HVIHH (SEQ. ID. NO: 22)
Glycine chloroplast Δ^{15}	VLGHDCGH (SEQ. ID. NO: 8)	HRTHH (SEQ. ID. NO: 14)	HVIHH (SEQ. ID. NO: 22)
Arab. fad3 (Δ^{15})	VLGHDCGH (SEQ. ID. NO: 8)	HRTHH (SEQ. ID. NO: 14)	HVIHH (SEQ. ID. NO: 22)
Brassica fad3 (Δ^{15})	VLGHDCGH (SEQ. ID. NO: 8)	HRTHH (SEQ. ID. NO: 14)	HVIHH (SEQ. ID. NO: 22)
Borage Δ^{12} (P1-81)*	VIAHECGH (SEQ. ID. NO: 9)	HRRHH (SEQ. ID. NO: 15)	HVAHH (SEQ. ID. NO: 23)
Arab. fad2 (Δ^{12})	VIAHECGH (SEQ. ID. NO: 9)	HRRHH (SEQ. ID. NO: 15)	HVAHH (SEQ. ID. NO: 23)
Arab. chloroplast Δ^{12}	VIGHDCAH (SEQ. ID. NO: 10)	HDRHH (SEQ. ID. NO: 16)	HIPHH (SEQ. ID. NO: 24)
Glycine plastid Δ^{12}	VIGHDCAH (SEQ. ID. NO: 10)	HDRHH (SEQ. ID. NO: 16)	HIPHH (SEQ. ID. NO: 24)
Spinach plastidial n-6	VIGHDCAH (SEQ. ID. NO: 10)	HDQHH (SEQ. ID. NO: 17)	HIPHH (SEQ. ID. NO: 24)
Synechocystis Δ^{12}	VVGHDCGH (SEQ. ID. NO: 11)	HDHHH (SEQ. ID. NO: 18)	HIPHH (SEQ. ID. NO: 24)
Anabaena Δ^{12}	VLGHDCGH (SEQ. ID. NO: 8)	HNHHH (SEQ. ID. NO: 19)	HVPHH (SEQ. ID. NO: 25)

*P1-81 is a full length cDNA which was identified by EST analysis and shows high similarity to the Arbidopsis Δ12 desaturase (fad2)

EXAMPLE 11

Construction of 121.Δ^6.NOS for stable transformation

The vector pBI121 (Jefferson et al. 1987 EMBO J. 6:3901-3907) was prepared for ligation by digestion with BamHI and EcoICR I (Promega) which excises the GUS coding region leaving the 35S promoter and NOS terminator intact. The borage Δ 6-desaturase cDNA was excised from the Bluescript plasmid (Stratagene) by digestion with BamHI and XhoI. The XhoI end was made blunt by use of the Klenow fragment. This fragment was then cloned into the BamHI/EcoICR I sites of pBI121, yielding 121.1Δ^6NOS (Fig. 7). In 121.Δ^6.NOS, the remaining portion (backbone) of the restriction map depicted in Fig. 7 is pBI121.

EXAMPLE 13

Stable transformation of tobacco

121.Δ^6.NOS plasmid construction was used to transform tobacco (Nicotiana tabacum cv. xanthi) via Agrobacterium according to standard procedures (Horsh et al., 1985 Science 227: 1229-1231; Bogue et al., 1990 Mol. Gen. Genet. 221:49-57), except that initial transformants were selected on 100 ug/ml kanamycin.

profile of seed tissue of a tobacco plant transformed with pBI 121Δ^6NOS. Peaks correspond to 18:2, 18:3γ(GLA) and 18:3α.

The relative distribution of the C_{18} fatty acids in control and transgenic tobacco seeds is shown in Table 4.

TABLE 4

Fatty Acid	Xanthi	pBI121Δ^6NOS
18:0	4.0%	2.5%
18:1	13%	13%
18:2	82%	82%
18:3γ(GLA)	-	2.7%
18:3α	0.82%	1.4%

The foregoing results demonstrate that GLA is incorporated into the triacylglycerides of transgenic tobacco leaves and seeds containing the borage Δ6-desaturase.

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 2002..3081

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCTAGCCACC AGTGACGATG CCTTGAATTT GGCCATTCTG ACCCAGGCCC GTATTCTGAA 60

TCCCCGCATT CGCATTGTTA ATCGTTTGTT CAACCATGCC CTGGGTAAAC GTTTAGACAC 120

CACCTTGCCA GACCACGTTA GTTTGAGTGT TTCCGCCCTG GCGGCCCCGA TTTTTTCCTT 180

TGCGGCTTTG GGCAATCAGG CGATCGGGCA ATTGCGTTTG TTTGACCAGA CTTGGCCCAT 240

TCAGGAAATT GTCATTCACC AAGACCATCC CTGGCTCAAT TTACCCCTGG CGGATTTATG 300

GGATGATCCG AGCCGAATGT TGATCTATTA CCTACCGGCC CACAGTGAAA CGGATTTAGT 360

AGGCGCAGTG GTGAATAATT TAACGTTGCA ATCTGGGGAC CATTTAATAG TGGGACAAAA 420

ACCCCAACCC AAGACCAAAC GGCGATCGCC TTGGCGCAAA TTTTCCAAAC TGATTACCAA 480

CCTGCGGGAG TATCAGCGGT ATGTCCAACA GGTGATATGG GTGGTGTTGT TTTTATTGTT 540

GATGATTTTT CTGGCCACCT TCATCTACGT TTCCATTGAT CAACATATTG CCCCAGTGGA 600

CGCGTTGTAT TTTTCCGTGG GCATGATTAC CGGGGCCGGT GGCAAGGAAG AGGTGGCCGA 660

AAAGTCCCCC GATATCATCA AAGTATTCAC AGTGGTGATG ATGATCGCCG GGGCGGGGGT 720

GATTGGTATT TGTTATGCCC TACTGAATGA TTTCATCCTT GGCAGTCGCT TTAGTCAGTT 780

TTTGGATGCG GCCAAGTTAC CCGATCGCCA TCACATCATC ATTTGTGGGC TGGGGGGAGT 840

GAGCATGGCC ATTATTGAAG AGTTAATTCA CCAGGGCCAT GAAATTGTGG TAATCGAAAA 900

GGATACAGAT AATCGTTTCT TGCATACGGC CCGCTCCCTG GGGGTGCCCG TAATTGTGGA 960

GGATGCCCGC CTAGAAAGAA CGTTGGCCTG CGCCAATATC AACCGAGCCG AAGCCATTGT 1020

GGTGGCCACC AGCGACGACA CCGTTAACTT GGAAATTGGC CTAACTGCCA AGGCGATCGC 1080

CCCTAGCCTG CCAGTGGTGT TGCGTTGCCA GGATGCCCAG TTTAGCCTGT CCCTGCAGGA 1140

AGTATTTGAA TTTGAAACGG TGCTTTGTCC GGCGGAATTG GCCACCTATT CCTTTGCGGC 1200

GGCGGCCCTG GGGGGCAAAA TTTTGGGCAA CGGCATGACC GATGATTTGC TGTGGGTAGC 1260

CCTAGCCACC TTAATCACTC CTAACCATCC CTTTGCCGAC CAATTGGTTA AAATTGCAGC 1320

CCAAAAGTCT GATTTCGTTC CCCTCTATCT AGAACGGGGT GGCAAAACCA TCCATAGCTG 1380

GGAATTATTG GGTACCCATC TCGACTCTGG AGACGTGTTG TATTTAACCA TGCCCGCCAC 1440

TGCCCTAGAG CAACTTTGGC GATCGCCCCG TGCCACTGCT GATCCTCTGG ACTCTTTTTT 1500

```
GTT GGT ATT TAT CGT TTC CAG CAA TTT TAT ATT TGG GGT TTA TAT CTT      2511
Val Gly Ile Tyr Arg Phe Gln Gln Phe Tyr Ile Trp Gly Leu Tyr Leu
155                 160                 165                 170

TTC ATT CCC TTT TAT TGG TTT CTC TAC GAT GTC TAC CTA GTG CTT AAT      2559
Phe Ile Pro Phe Tyr Trp Phe Leu Tyr Asp Val Tyr Leu Val Leu Asn
                175                 180                 185

AAA GGC AAA TAT CAC GAC CAT AAA ATT CCT CCT TTC CAG CCC CTA GAA      2607
Lys Gly Lys Tyr His Asp His Lys Ile Pro Pro Phe Gln Pro Leu Glu
            190                 195                 200

TTA GCT AGT TTG CTA GGG ATT AAG CTA TTA TGG CTC GGC TAC GTT TTC      2655
Leu Ala Ser Leu Leu Gly Ile Lys Leu Leu Trp Leu Gly Tyr Val Phe
        205                 210                 215

GGC TTA CCT CTG GCT CTG GGC TTT TCC ATT CCT GAA GTA TTA ATT GGT      2703
Gly Leu Pro Leu Ala Leu Gly Phe Ser Ile Pro Glu Val Leu Ile Gly
    220                 225                 230

GCT TCG GTA ACC TAT ATG ACC TAT GGC ATC GTG GTT TGC ACC ATC TTT      2751
Ala Ser Val Thr Tyr Met Thr Tyr Gly Ile Val Val Cys Thr Ile Phe
235                 240                 245                 250

ATG CTG GCC CAT GTG TTG GAA TCA ACT GAA TTT CTC ACC CCC GAT GGT      2799
Met Leu Ala His Val Leu Glu Ser Thr Glu Phe Leu Thr Pro Asp Gly
                255                 260                 265

GAA TCC GGT GCC ATT GAT GAC GAG TGG GCT ATT TGC CAA ATT CGT ACC      2847
Glu Ser Gly Ala Ile Asp Asp Glu Trp Ala Ile Cys Gln Ile Arg Thr
            270                 275                 280

ACG GCC AAT TTT GCC ACC AAT AAT CCC TTT TGG AAC TGG TTT TGT GGC      2895
Thr Ala Asn Phe Ala Thr Asn Asn Pro Phe Trp Asn Trp Phe Cys Gly
        285                 290                 295

GGT TTA AAT CAC CAA GTT ACC CAC CAT CTT TTC CCC AAT ATT TGT CAT      2943
Gly Leu Asn His Gln Val Thr His His Leu Phe Pro Asn Ile Cys His
    300                 305                 310

ATT CAC TAT CCC CAA TTG GAA AAT ATT ATT AAG GAT GTT TGC CAA GAG      2991
Ile His Tyr Pro Gln Leu Glu Asn Ile Ile Lys Asp Val Cys Gln Glu
315                 320                 325                 330

TTT GGT GTG GAA TAT AAA GTT TAT CCC ACC TTC AAA GCG GCG ATC GCC      3039
Phe Gly Val Glu Tyr Lys Val Tyr Pro Thr Phe Lys Ala Ala Ile Ala
                335                 340                 345

TCT AAC TAT CGC TGG CTA GAG GCC ATG GGC AAA GCA TCG TGACATTGCC       3088
Ser Asn Tyr Arg Trp Leu Glu Ala Met Gly Lys Ala Ser
            350                 355                 360

TTGGGATTGA AGCAAAATGG CAAAATCCCT CGTAAATCTA TGATCGAAGC CTTTCTGTTG    3148

CCCGCCGACC AAATCCCCGA TGCTGACCAA AGGTTGATGT TGGCATTGCT CCAAACCCAC    3208
```

Gln Gln Phe Tyr Ile Trp Gly Leu Tyr Leu Phe Ile Pro Phe Tyr Trp
165 170 175

Phe Leu Tyr Asp Val Tyr Leu Val Leu Asn Lys Gly Lys Tyr His Asp
180 185 190

His Lys Ile Pro Pro Phe Gln Pro Leu Glu Leu Ala Ser Leu Leu Gly
195 200 205

Ile Lys Leu Leu Trp Leu Gly Tyr Val Phe Gly Leu Pro Leu Ala Leu
210 215 220

Gly Phe Ser Ile Pro Glu Val Leu Ile Gly Ala Ser Val Thr Tyr Met
225 230 235 240

Thr Tyr Gly Ile Val Val Cys Thr Ile Phe Met Leu Ala His Val Leu
245 250 255

Glu Ser Thr Glu Phe Leu Thr Pro Asp Gly Glu Ser Gly Ala Ile Asp
260 265 270

Asp Glu Trp Ala Ile Cys Gln Ile Arg Thr Thr Ala Asn Phe Ala Thr
275 280 285

Asn Asn Pro Phe Trp Asn Trp Phe Cys Gly Gly Leu Asn His Gln Val
290 295 300

Thr His His Leu Phe Pro Asn Ile Cys His Ile His Tyr Pro Gln Leu
305 310 315 320

Glu Asn Ile Ile Lys Asp Val Cys Gln Glu Phe Gly Val Glu Tyr Lys
325 330 335

Val Tyr Pro Thr Phe Lys Ala Ala Ile Ala Ser Asn Tyr Arg Trp Leu
340 345 350

Glu Ala Met Gly Lys Ala Ser
355

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1884 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AGCTTCACTT CGGTTTTATA TTGTGACCAT GGTTCCCAGG CATCTGCTCT AGGGAGTTTT 60

TCCGCTGCCT TTAGAGAGTA TTTTCTCCAA GTCGGCTAAC TCCCCCATTT TTAGGCAAAA 120

TTTGAGCATT TTTGCCAAGG AATTCTATCC CCACTATCTC CATCCCACTC CCCCGCCTGT 1860

ACAAAATTTT ATCCATCAGC TAGC 1884

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1685 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AATATCTGCC TACCCTCCCA AAGAGAGTAG TCATTTTTCA TCAATGGCTG CTCAAATCAA 60

GAAATACATT ACCTCAGATG AACTCAAGAA CCACGATAAA CCCGGAGATC TATGGATCTC 120

GATTCAAGGG AAAGCCTATG ATGTTTCGGA TTGGGTGAAA GACCATCCAG GTGGCAGCTT 180

TCCCTTGAAG AGTCTTGCTG GTCAAGAGGT AACTGATGCA TTTGTTGCAT TCCATCCTGC 240

CTCTACATGG AAGAATCTTG ATAAGTTTTT CACTGGGTAT TATCTTAAAG ATTACTCTGT 300

TTCTGAGGTT TCTAAAGATT ATAGGAAGCT TGTGTTTGAG TTTTCTAAAA TGGGTTTGTA 360

TGACAAAAAA GGTCATATTA TGTTTGCAAC TTTGTGCTTT ATAGCAATGC TGTTTGCTAT 420

GAGTGTTTAT GGGGTTTTGT TTTGTGAGGG TGTTTTGGTA CATTTGTTTT CTGGGTGTTT 480

GATGGGGTTT CTTTGGATTC AGAGTGGTTG GATTGGACAT GATGCTGGGC ATTATATGGT 540

AGTGTCTGAT TCAAGGCTTA ATAAGTTTAT GGGTATTTTT GCTGCAAATT GTCTTTCAGG 600

AATAAGTATT GGTTGGTGGA AATGGAACCA TAATGCACAT CACATTGCCT GTAATAGCCT 660

TGAATATGAC CCTGATTTAC AATATATACC ATTCCTTGTT GTGTCTTCCA AGTTTTTTGG 720

TTCACTCACC TCTCATTTCT ATGAGAAAAG GTTGACTTTT GACTCTTTAT CAAGATTCTT 780

TGTAAGTTAT CAACATTGGA CATTTTACCC TATTATGTGT GCTGCTAGGC TCAATATGTA 840

TGTACAATCT CTCATAATGT TGTTGACCAA GAGAAATGTG TCCTATCGAG CTCAGGAACT 900

CTTGGGATGC CTAGTGTTCT CGATTTGGTA CCCGTTGCTT GTTTCTTGTT TGCCTAATTG 960

GGGTGAAAGA ATTATGTTTG TTATTGCAAG TTTATCAGTG ACTGGAATGC AACAAGTTCA 1020

GTTCTCCTTG AACCACTTCT CTTCAAGTGT TTATGTTGGA AAGCCTAAAG GGAATAATTG 1080

GTTTGAGAAA CAAACGGATG GGACACTTGA CATTTCTTGT CCTCCTTGGA TGGATTGGTT 1140

TCATGGTGGA TTGCAATTCC AAATTGAGCA TCATTTGTTT CCCAAGATGC CTAGATGCAA 1200

Cys Leu Met Gly Phe Leu Trp Ile Gln Ser Gly Trp Ile Gly His Asp
145 150 155 160

Ala Gly His Tyr Met Val Val Ser Asp Ser Arg Leu Asn Lys Phe Met
165 170 175

Gly Ile Phe Ala Ala Asn Cys Leu Ser Gly Ile Ser Ile Gly Trp Trp
180 185 190

Lys Trp Asn His Asn Ala His His Ile Ala Cys Asn Ser Leu Glu Tyr
195 200 205

Asp Pro Asp Leu Gln Tyr Ile Pro Phe Leu Val Val Ser Ser Lys Phe
210 215 220

Phe Gly Ser Leu Thr Ser His Phe Tyr Glu Lys Arg Leu Thr Phe Asp
225 230 235 240

Ser Leu Ser Arg Phe Phe Val Ser Tyr Gln His Trp Thr Phe Tyr Pro
245 250 255

Ile Met Cys Ala Ala Arg Leu Asn Met Tyr Val Gln Ser Leu Ile Met
260 265 270

Leu Leu Thr Lys Arg Asn Val Ser Tyr Arg Ala Gln Glu Leu Leu Gly
275 280 285

Cys Leu Val Phe Ser Ile Trp Tyr Pro Leu Leu Val Ser Cys Leu Pro
290 295 300

Asn Trp Gly Glu Arg Ile Met Phe Val Ile Ala Ser Leu Ser Val Thr
305 310 315 320

Gly Met Gln Gln Val Gln Phe Ser Leu Asn His Phe Ser Ser Ser Val
325 330 335

Tyr Val Gly Lys Pro Lys Gly Asn Asn Trp Phe Glu Lys Gln Thr Asp
340 345 350

Gly Thr Leu Asp Ile Ser Cys Pro Pro Trp Met Asp Trp Phe His Gly
355 360 365

Gly Ser Gln Phe Gln Ile Glu His His Leu Phe Pro Lys Met Pro Arg
370 375 380

Cys Asn Leu Arg Lys Ile Ser Pro Tyr Val Ile Glu Leu Cys Lys Lys
385 390 395 400

His Asn Leu Pro Tyr Asn Tyr Ala Ser Phe Ser Lys Ala Asn Glu Met
405 410 415

Thr Leu Arg Thr Leu Arg Asn Thr Ala Leu Gln Ala Arg Asp Ile Thr
420 425 430

Lys Pro Leu Pro Lys Asn Leu Val Trp Glu Ala Leu His Thr His Gly
435 440 445

Val Ile Ala His Glu Cys Gly His
1 5

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Val Ile Gly His Asp Cys Ala His
1 5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Val Val Gly His Asp Cys Gly His
1 5

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

His Asn Ala His His
1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

His Asp Arg His His
1 5

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

His Asp Gln His His
1 5

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

His Asp His His His
1 5

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

His Val Ala His His
1 5

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

His Ile Pro His His
1 5

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

His Val Pro His His
1 5

signal, a nopaline synthase termination signal, or a seed termination signal.

9. A cell comprising the vector of any one of Claims 4-8.

10. The cell of Claim 9 wherein said cell is an animal cell, a bacterial cell, a plant cell or a fungal cell.

11. A transgenic organism comprising the isolated nucleic acid of any one of Claims 1-3.

12. A transgenic organism comprising the vector of any one of Claims 4-8.

13. The transgenic organism of Claim 11 or 12 wherein said organism is a bacterium, a fungus, a plant or an animal.

14. A plant or progeny of said plant which has been regenerated from the plant cell of Claim 10.

15. The plant of Claim 14 wherein said plant is a sunflower, soybean, maize, tobacco, peanut, carrot or oil seed rape plant.

16. A method of producing a plant with increased gamma linolenic acid (GLA) content which comprises:

borage Δ6-desaturase and an isolated nucleic acid encoding Δ12-desaturase.

22. The method of Claim 21 wherein said isolated nucleic acid encoding Δ6-desaturase comprises nucleotides 44 to 1390 of SEQ. ID NO: 4.

23. A method of inducing production of octadecatetraeonic acid in an organism deficient or lacking in gamma linolenic acid which comprises transforming said organism with the isolated nucleic acid of any one of Claims 1-3.

24. A method of inducing production of octadecatetraeonic acid in an organism deficient or lacking in gamma linolenic acid which comprises transforming said organism with the vector of any one of Claims 4-8.

25. The method of Claim 23 or 24 wherein said organism is a bacterium, a fungus, a plant or an animal.

26. A method of producing a plant with improved chilling resistance which comprises:

(a) transforming a plant cell with the isolated nucleic acid of any one of Claims 1-3; and

(b) regenerating said plant with improved chilling resistance from said transformed plant cell.

27. A method of producing a plant with improved chilling resistance which comprises:

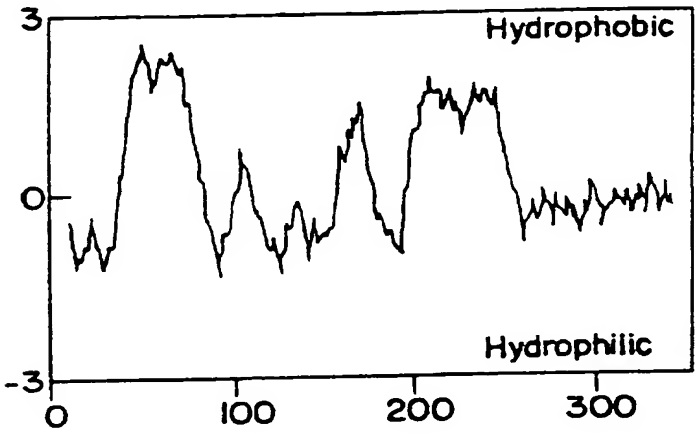

3
Hydrophobic
0
Hydrophilic
-3
0
100
200
300

FIG. 1A

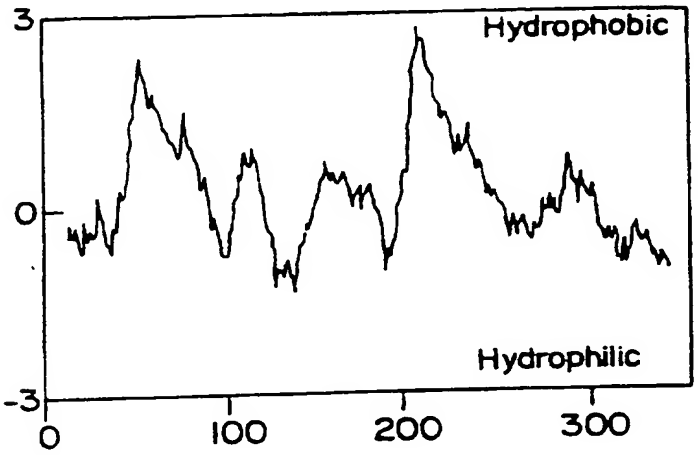

3
Hydrophobic
0
Hydrophilic
-3
0
100
200
300

FIG. 1B

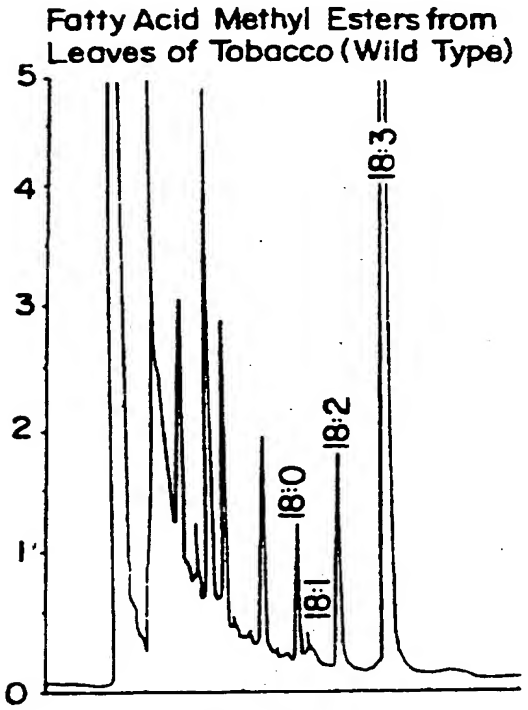

Fatty Acid Methyl Esters from
Leaves of Tobacco (Wild Type)
5
4
3
2
1
0
18:0
18:1
18:2
18:3

FIG. 4A

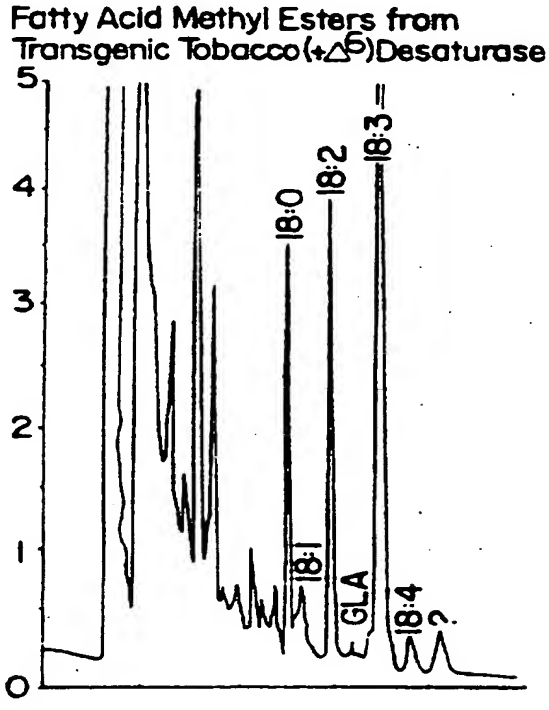

Fatty Acid Methyl Esters from
Transgenic Tobacco (+Δ6) Desaturase
5
4
3
2
1
0
18:0
18:2
18:3
18:1
GLA
18:4
?

FIG. 4B

FIG. 6

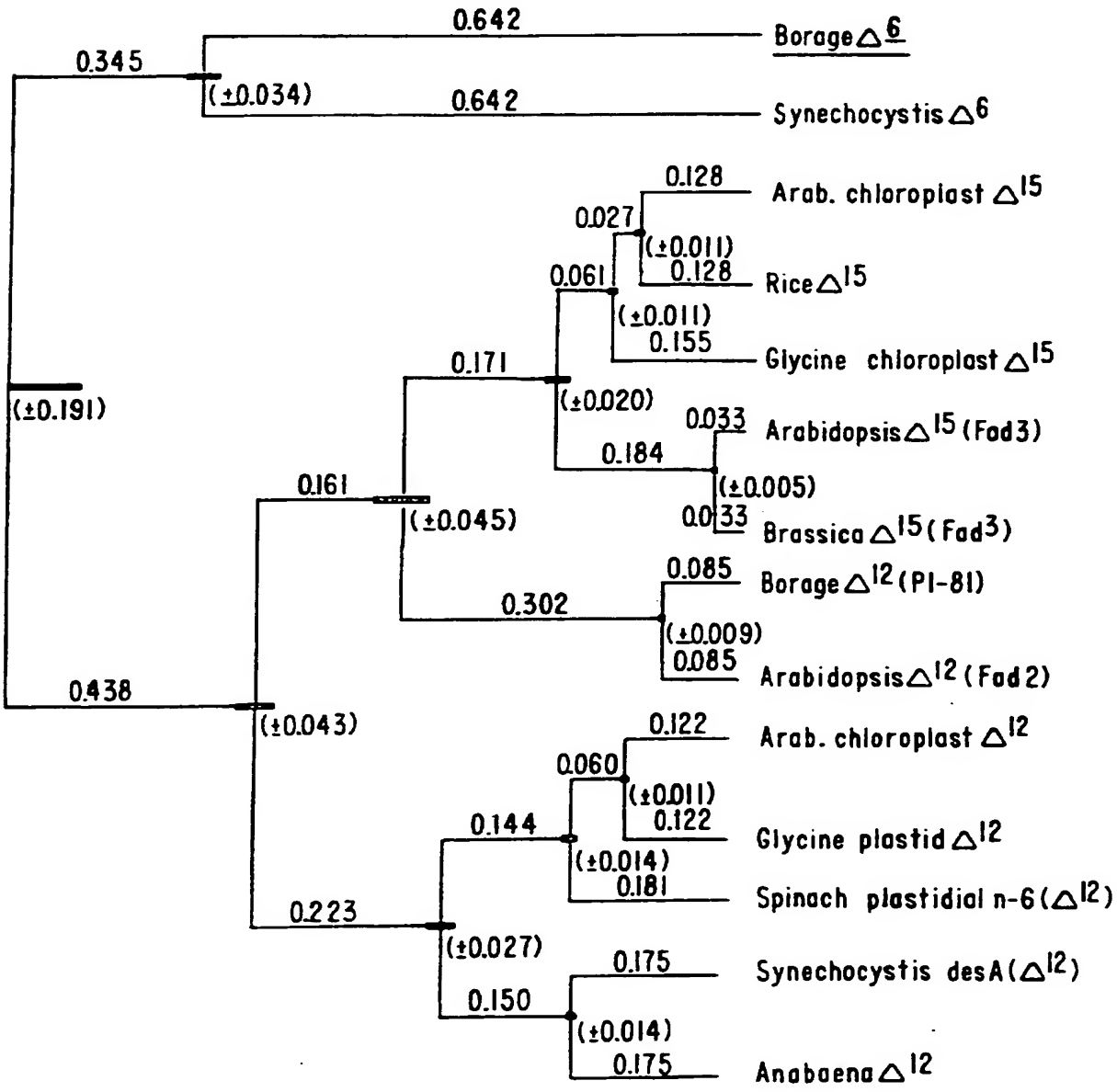

0.642
Borage Δ6
0.345
(±0.034)
0.642
Synechocystis Δ6
0.128
Arab. chloroplast Δ15
0.027
(±0.011)
0.061
0.128
Rice Δ15
(±0.011)
0.155
Glycine chloroplast Δ15
0.171
(±0.191)
(±0.020)
0.033
Arabidopsis Δ15 (Fad3)
0.184
(±0.005)
0.161
(±0.045)
0.033
Brassica Δ15 (Fad3)
0.085
Borage Δ12 (Pl-81)
0.302
(±0.009)
0.085
Arabidopsis Δ12 (Fad2)
0.438
(±0.043)
0.122
Arab. chloroplast Δ12
0.060
(±0.011)
0.144
0.122
Glycine plastid Δ12
(±0.014)
0.181
Spinach plastidial n-6 (Δ12)
0.223
(±0.027)
0.175
Synechocystis desA (Δ12)
0.150
(±0.014)
0.175
Anabaena Δ12

FIG. 8A

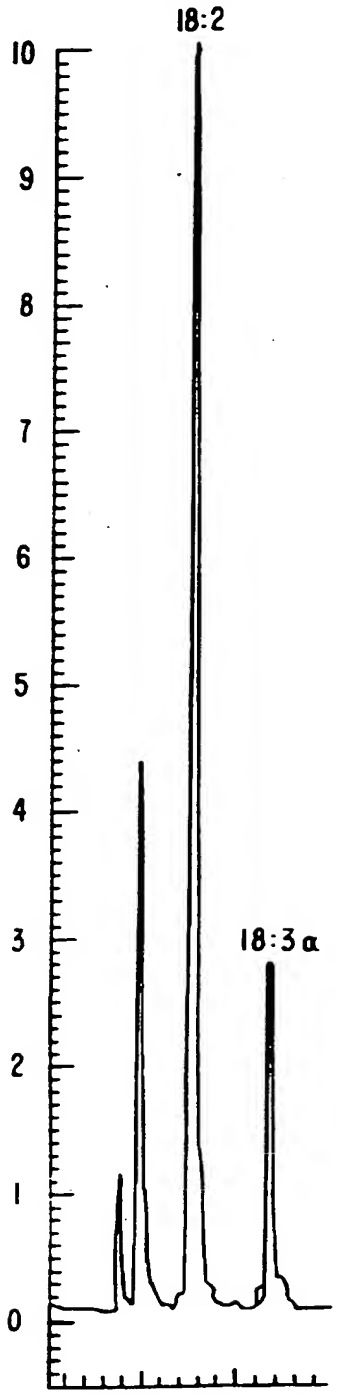


FIG. 8B

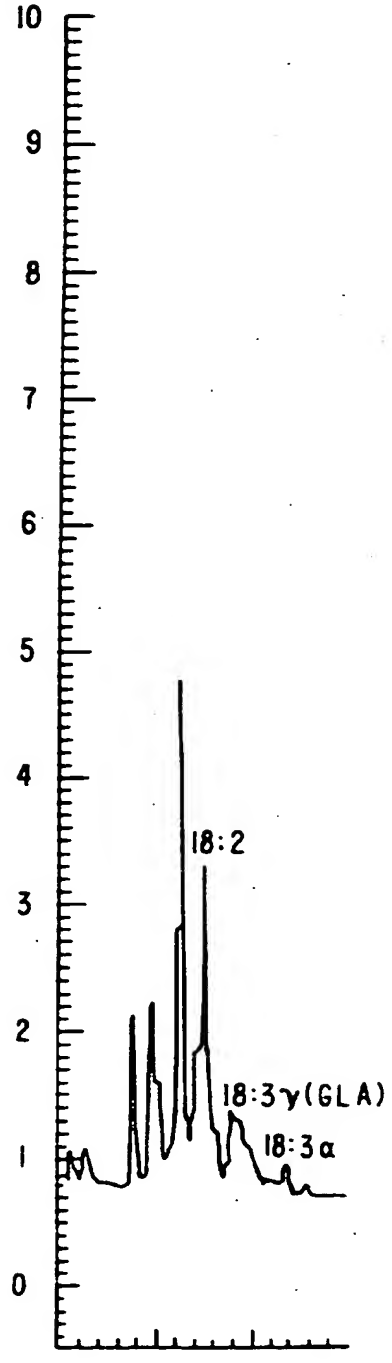


FIG. 10A

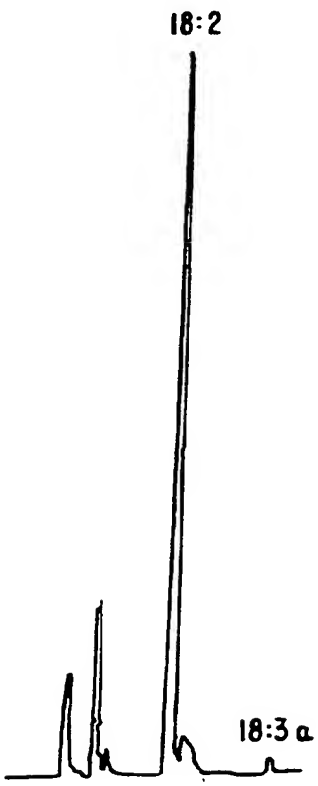
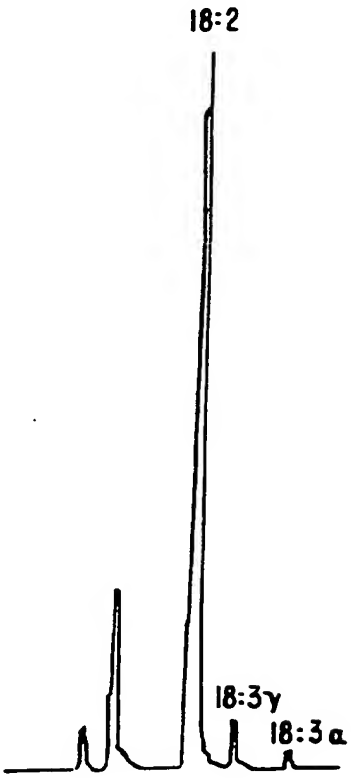


FIG. 10B



EXAMPLE 11

Construction of 121.Δ^6.NOS for stable transformation

The vector pBI121 (Jefferson et al. 1987 EMBO J. 6:3901-3907) was prepared for ligation by digestion with BamHI and EcoICR I (Promega) which excises the GUS coding region leaving the 35S promoter and NOS terminator intact. The borage Δ 6-desaturase cDNA was excised from the Bluescript plasmid (Stratagene) by digestion with BamHI and XhoI. The XhoI end was made blunt by use of the Klenow fragment. This fragment was then cloned into the BamHI/EcoICR I sites of pBI121, yielding 121.1Δ^6NOS (Fig. 7). In 121.Δ^6.NOS, the remaining portion (backbone) of the restriction map depicted in Fig. 7 is pBI121.

EXAMPLE 13

Stable transformation of tobacco

121.Δ^6.NOS plasmid construction was used to transform tobacco (Nicotiana tabacum cv. xanthi) via Agrobacterium according to standard procedures (Horsh et al., 1985 Science 227: 1229-1231; Bogue et al., 1990 Mol. Gen. Genet. 221:49-57), except that initial transformants were selected on 100 ug/ml kanamycin.

profile of seed tissue of a tobacco plant transformed with pBI 121Δ^6NOS. Peaks correspond to 18:2, 18:3γ(GLA) and 18:3α.

The relative distribution of the C_{18} fatty acids in control and transgenic tobacco seeds is shown in Table 4.

TABLE 4

Fatty Acid	Xanthi	pBI121Δ^6NOS
18:0	4.0%	2.5%
18:1	13%	13%
18:2	82%	82%
18:3γ(GLA)	-	2.7%
18:3α	0.82%	1.4%

The foregoing results demonstrate that GLA is incorporated into the triacylglycerides of transgenic tobacco leaves and seeds containing the borage Δ6-desaturase.

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 2002..3081

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCTAGCCACC AGTGACGATG CCTTGAATTT GGCCATTCTG ACCCAGGCCC GTATTCTGAA 60

TCCCCGCATT CGCATTGTTA ATCGTTTGTT CAACCATGCC CTGGGTAAAC GTTTAGACAC 120

CACCTTGCCA GACCACGTTA GTTTGAGTGT TTCCGCCCTG GCGGCCCCGA TTTTTTCCTT 180

TGCGGCTTTG GGCAATCAGG CGATCGGGCA ATTGCGTTTG TTTGACCAGA CTTGGCCCAT 240

TCAGGAAATT GTCATTCACC AAGACCATCC CTGGCTCAAT TTACCCCTGG CGGATTTATG 300

GGATGATCCG AGCCGAATGT TGATCTATTA CCTACCGGCC CACAGTGAAA CGGATTTAGT 360

AGGCGCAGTG GTGAATAATT TAACGTTGCA ATCTGGGGAC CATTTAATAG TGGGACAAAA 420

ACCCCAACCC AAGACCAAAC GGCGATCGCC TTGGCGCAAA TTTTCCAAAC TGATTACCAA 480

CCTGCGGGAG TATCAGCGGT ATGTCCAACA GGTGATATGG GTGGTGTTGT TTTTATTGTT 540

GATGATTTTT CTGGCCACCT TCATCTACGT TTCCATTGAT CAACATATTG CCCCAGTGGA 600

CGCGTTGTAT TTTTCCGTGG GCATGATTAC CGGGGCCGGT GGCAAGGAAG AGGTGGCCGA 660

AAAGTCCCCC GATATCATCA AAGTATTCAC AGTGGTGATG ATGATCGCCG GGGCGGGGGT 720

GATTGGTATT TGTTATGCCC TACTGAATGA TTTCATCCTT GGCAGTCGCT TTAGTCAGTT 780

TTTGGATGCG GCCAAGTTAC CCGATCGCCA TCACATCATC ATTTGTGGGC TGGGGGGAGT 840

GAGCATGGCC ATTATTGAAG AGTTAATTCA CCAGGGCCAT GAAATTGTGG TAATCGAAAA 900

GGATACAGAT AATCGTTTCT TGCATACGGC CCGCTCCCTG GGGGTGCCCG TAATTGTGGA 960

GGATGCCCGC CTAGAAAGAA CGTTGGCCTG CGCCAATATC AACCGAGCCG AAGCCATTGT 1020

GGTGGCCACC AGCGACGACA CCGTTAACTT GGAAATTGGC CTAACTGCCA AGGCGATCGC 1080

CCCTAGCCTG CCAGTGGTGT TGCGTTGCCA GGATGCCCAG TTTAGCCTGT CCCTGCAGGA 1140

AGTATTTGAA TTTGAAACGG TGCTTTGTCC GGCGGAATTG GCCACCTATT CCTTTGCGGC 1200

GGCGGCCCTG GGGGGCAAAA TTTTGGGCAA CGGCATGACC GATGATTTGC TGTGGGTAGC 1260

CCTAGCCACC TTAATCACTC CTAACCATCC CTTTGCCGAC CAATTGGTTA AAATTGCAGC 1320

CCAAAAGTCT GATTTCGTTC CCCTCTATCT AGAACGGGGT GGCAAAACCA TCCATAGCTG 1380

GGAATTATTG GGTACCCATC TCGACTCTGG AGACGTGTTG TATTTAACCA TGCCCGCCAC 1440

TGCCCTAGAG CAACTTTGGC GATCGCCCCG TGCCACTGCT GATCCTCTGG ACTCTTTTTT 1500

```
GTT GGT ATT TAT CGT TTC CAG CAA TTT TAT ATT TGG GGT TTA TAT CTT      2511
Val Gly Ile Tyr Arg Phe Gln Gln Phe Tyr Ile Trp Gly Leu Tyr Leu
155                 160                 165                 170

TTC ATT CCC TTT TAT TGG TTT CTC TAC GAT GTC TAC CTA GTG CTT AAT      2559
Phe Ile Pro Phe Tyr Trp Phe Leu Tyr Asp Val Tyr Leu Val Leu Asn
                175                 180                 185

AAA GGC AAA TAT CAC GAC CAT AAA ATT CCT CCT TTC CAG CCC CTA GAA      2607
Lys Gly Lys Tyr His Asp His Lys Ile Pro Pro Phe Gln Pro Leu Glu
            190                 195                 200

TTA GCT AGT TTG CTA GGG ATT AAG CTA TTA TGG CTC GGC TAC GTT TTC      2655
Leu Ala Ser Leu Leu Gly Ile Lys Leu Leu Trp Leu Gly Tyr Val Phe
        205                 210                 215

GGC TTA CCT CTG GCT CTG GGC TTT TCC ATT CCT GAA GTA TTA ATT GGT      2703
Gly Leu Pro Leu Ala Leu Gly Phe Ser Ile Pro Glu Val Leu Ile Gly
    220                 225                 230

GCT TCG GTA ACC TAT ATG ACC TAT GGC ATC GTG GTT TGC ACC ATC TTT      2751
Ala Ser Val Thr Tyr Met Thr Tyr Gly Ile Val Val Cys Thr Ile Phe
235                 240                 245                 250

ATG CTG GCC CAT GTG TTG GAA TCA ACT GAA TTT CTC ACC CCC GAT GGT      2799
Met Leu Ala His Val Leu Glu Ser Thr Glu Phe Leu Thr Pro Asp Gly
                255                 260                 265

GAA TCC GGT GCC ATT GAT GAC GAG TGG GCT ATT TGC CAA ATT CGT ACC      2847
Glu Ser Gly Ala Ile Asp Asp Glu Trp Ala Ile Cys Gln Ile Arg Thr
            270                 275                 280

ACG GCC AAT TTT GCC ACC AAT AAT CCC TTT TGG AAC TGG TTT TGT GGC      2895
Thr Ala Asn Phe Ala Thr Asn Asn Pro Phe Trp Asn Trp Phe Cys Gly
        285                 290                 295

GGT TTA AAT CAC CAA GTT ACC CAC CAT CTT TTC CCC AAT ATT TGT CAT      2943
Gly Leu Asn His Gln Val Thr His His Leu Phe Pro Asn Ile Cys His
    300                 305                 310

ATT CAC TAT CCC CAA TTG GAA AAT ATT ATT AAG GAT GTT TGC CAA GAG      2991
Ile His Tyr Pro Gln Leu Glu Asn Ile Ile Lys Asp Val Cys Gln Glu
315                 320                 325                 330

TTT GGT GTG GAA TAT AAA GTT TAT CCC ACC TTC AAA GCG GCG ATC GCC      3039
Phe Gly Val Glu Tyr Lys Val Tyr Pro Thr Phe Lys Ala Ala Ile Ala
                335                 340                 345

TCT AAC TAT CGC TGG CTA GAG GCC ATG GGC AAA GCA TCG TGACATTGCC       3088
Ser Asn Tyr Arg Trp Leu Glu Ala Met Gly Lys Ala Ser
            350                 355                 360

TTGGGATTGA AGCAAAATGG CAAAATCCCT CGTAAATCTA TGATCGAAGC CTTTCTGTTG    3148

CCCGCCGACC AAATCCCCGA TGCTGACCAA AGGTTGATGT TGGCATTGCT CCAAACCCAC    3208
```

```
Gln Gln Phe Tyr Ile Trp Gly Leu Tyr Leu Phe Ile Pro Phe Tyr Trp
                165                 170                 175

Phe Leu Tyr Asp Val Tyr Leu Val Leu Asn Lys Gly Lys Tyr His Asp
            180                 185                 190

His Lys Ile Pro Pro Phe Gln Pro Leu Glu Leu Ala Ser Leu Leu Gly
        195                 200                 205

Ile Lys Leu Leu Trp Leu Gly Tyr Val Phe Gly Leu Pro Leu Ala Leu
    210                 215                 220

Gly Phe Ser Ile Pro Glu Val Leu Ile Gly Ala Ser Val Thr Tyr Met
225                 230                 235                 240

Thr Tyr Gly Ile Val Val Cys Thr Ile Phe Met Leu Ala His Val Leu
                245                 250                 255

Glu Ser Thr Glu Phe Leu Thr Pro Asp Gly Glu Ser Gly Ala Ile Asp
            260                 265                 270

Asp Glu Trp Ala Ile Cys Gln Ile Arg Thr Thr Ala Asn Phe Ala Thr
        275                 280                 285

Asn Asn Pro Phe Trp Asn Trp Phe Cys Gly Gly Leu Asn His Gln Val
    290                 295                 300

Thr His His Leu Phe Pro Asn Ile Cys His Ile His Tyr Pro Gln Leu
305                 310                 315                 320

Glu Asn Ile Ile Lys Asp Val Cys Gln Glu Phe Gly Val Glu Tyr Lys
                325                 330                 335

Val Tyr Pro Thr Phe Lys Ala Ala Ile Ala Ser Asn Tyr Arg Trp Leu
            340                 345                 350

Glu Ala Met Gly Lys Ala Ser
        355
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1884 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
AGCTTCACTT CGGTTTTATA TTGTGACCAT GGTTCCCAGG CATCTGCTCT AGGGAGTTTT      60

TCCGCTGCCT TTAGAGAGTA TTTTCTCCAA GTCGGCTAAC TCCCCCATTT TTAGGCAAAA     120
```

TTTGAGCATT TTTGCCAAGG AATTCTATCC CCACTATCTC CATCCCACTC CCCCGCCTGT 1860

ACAAAATTTT ATCCATCAGC TAGC 1884

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1685 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AATATCTGCC TACCCTCCCA AAGAGAGTAG TCATTTTTCA TCAATGGCTG CTCAAATCAA 60

GAAATACATT ACCTCAGATG AACTCAAGAA CCACGATAAA CCCGGAGATC TATGGATCTC 120

GATTCAAGGG AAAGCCTATG ATGTTTCGGA TTGGGTGAAA GACCATCCAG GTGGCAGCTT 180

TCCCTTGAAG AGTCTTGCTG GTCAAGAGGT AACTGATGCA TTTGTTGCAT TCCATCCTGC 240

CTCTACATGG AAGAATCTTG ATAAGTTTTT CACTGGGTAT TATCTTAAAG ATTACTCTGT 300

TTCTGAGGTT TCTAAAGATT ATAGGAAGCT TGTGTTTGAG TTTTCTAAAA TGGGTTTGTA 360

TGACAAAAAA GGTCATATTA TGTTTGCAAC TTTGTGCTTT ATAGCAATGC TGTTTGCTAT 420

GAGTGTTTAT GGGGTTTTGT TTTGTGAGGG TGTTTTGGTA CATTTGTTTT CTGGGTGTTT 480

GATGGGGTTT CTTTGGATTC AGAGTGGTTG GATTGGACAT GATGCTGGGC ATTATATGGT 540

AGTGTCTGAT TCAAGGCTTA ATAAGTTTAT GGGTATTTTT GCTGCAAATT GTCTTTCAGG 600

AATAAGTATT GGTTGGTGGA AATGGAACCA TAATGCACAT CACATTGCCT GTAATAGCCT 660

TGAATATGAC CCTGATTTAC AATATATACC ATTCCTTGTT GTGTCTTCCA AGTTTTTTGG 720

TTCACTCACC TCTCATTTCT ATGAGAAAAG GTTGACTTTT GACTCTTTAT CAAGATTCTT 780

TGTAAGTTAT CAACATTGGA CATTTTACCC TATTATGTGT GCTGCTAGGC TCAATATGTA 840

TGTACAATCT CTCATAATGT TGTTGACCAA GAGAAATGTG TCCTATCGAG CTCAGGAACT 900

CTTGGGATGC CTAGTGTTCT CGATTTGGTA CCCGTTGCTT GTTTCTTGTT TGCCTAATTG 960

GGGTGAAAGA ATTATGTTTG TTATTGCAAG TTTATCAGTG ACTGGAATGC AACAAGTTCA 1020

GTTCTCCTTG AACCACTTCT CTTCAAGTGT TTATGTTGGA AAGCCTAAAG GGAATAATTG 1080

GTTTGAGAAA CAAACGGATG GGACACTTGA CATTTCTTGT CCTCCTTGGA TGGATTGGTT 1140

TCATGGTGGA TTGCAATTCC AAATTGAGCA TCATTTGTTT CCCAAGATGC CTAGATGCAA 1200

Cys Leu Met Gly Phe Leu Trp Ile Gln Ser Gly Trp Ile Gly His Asp
145 150 155 160

Ala Gly His Tyr Met Val Val Ser Asp Ser Arg Leu Asn Lys Phe Met
165 170 175

Gly Ile Phe Ala Ala Asn Cys Leu Ser Gly Ile Ser Ile Gly Trp Trp
180 185 190

Lys Trp Asn His Asn Ala His His Ile Ala Cys Asn Ser Leu Glu Tyr
195 200 205

Asp Pro Asp Leu Gln Tyr Ile Pro Phe Leu Val Val Ser Ser Lys Phe
210 215 220

Phe Gly Ser Leu Thr Ser His Phe Tyr Glu Lys Arg Leu Thr Phe Asp
225 230 235 240

Ser Leu Ser Arg Phe Phe Val Ser Tyr Gln His Trp Thr Phe Tyr Pro
245 250 255

Ile Met Cys Ala Ala Arg Leu Asn Met Tyr Val Gln Ser Leu Ile Met
260 265 270

Leu Leu Thr Lys Arg Asn Val Ser Tyr Arg Ala Gln Glu Leu Leu Gly
275 280 285

Cys Leu Val Phe Ser Ile Trp Tyr Pro Leu Leu Val Ser Cys Leu Pro
290 295 300

Asn Trp Gly Glu Arg Ile Met Phe Val Ile Ala Ser Leu Ser Val Thr
305 310 315 320

Gly Met Gln Gln Val Gln Phe Ser Leu Asn His Phe Ser Ser Ser Val
325 330 335

Tyr Val Gly Lys Pro Lys Gly Asn Asn Trp Phe Glu Lys Gln Thr Asp
340 345 350

Gly Thr Leu Asp Ile Ser Cys Pro Pro Trp Met Asp Trp Phe His Gly
355 360 365

Gly Ser Gln Phe Gln Ile Glu His His Leu Phe Pro Lys Met Pro Arg
370 375 380

Cys Asn Leu Arg Lys Ile Ser Pro Tyr Val Ile Glu Leu Cys Lys Lys
385 390 395 400

His Asn Leu Pro Tyr Asn Tyr Ala Ser Phe Ser Lys Ala Asn Glu Met
405 410 415

Thr Leu Arg Thr Leu Arg Asn Thr Ala Leu Gln Ala Arg Asp Ile Thr
420 425 430

Lys Pro Leu Pro Lys Asn Leu Val Trp Glu Ala Leu His Thr His Gly
435 440 445

Val Ile Ala His Glu Cys Gly His
1 5

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Val Ile Gly His Asp Cys Ala His
1 5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Val Val Gly His Asp Cys Gly His
1 5

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

His Asn Ala His His
1 5

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

His Asp Arg His His
1 5

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

His Asp Gln His His
1 5

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

His Asp His His His
1 5

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

His Val Ala His His
1 5

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

His Ile Pro His His
1 5

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

His Val Pro His His
1 5

signal, a nopaline synthase termination signal, or a seed termination signal.

9. A cell comprising the vector of any one of Claims 4-8.

10. The cell of Claim 9 wherein said cell is an animal cell, a bacterial cell, a plant cell or a fungal cell.

11. A transgenic organism comprising the isolated nucleic acid of any one of Claims 1-3.

12. A transgenic organism comprising the vector of any one of Claims 4-8.

13. The transgenic organism of Claim 11 or 12 wherein said organism is a bacterium, a fungus, a plant or an animal.

14. A plant or progeny of said plant which has been regenerated from the plant cell of Claim 10.

15. The plant of Claim 14 wherein said plant is a sunflower, soybean, maize, tobacco, peanut, carrot or oil seed rape plant.

16. A method of producing a plant with increased gamma linolenic acid (GLA) content which comprises:

borage Δ6-desaturase and an isolated nucleic acid encoding Δ12-desaturase.

22. The method of Claim 21 wherein said isolated nucleic acid encoding Δ6-desaturase comprises nucleotides 44 to 1390 of SEQ. ID NO: 4.

23. A method of inducing production of octadecatetraeonic acid in an organism deficient or lacking in gamma linolenic acid which comprises transforming said organism with the isolated nucleic acid of any one of Claims 1-3.

24. A method of inducing production of octadecatetraeonic acid in an organism deficient or lacking in gamma linolenic acid which comprises transforming said organism with the vector of any one of Claims 4-8.

25. The method of Claim 23 or 24 wherein said organism is a bacterium, a fungus, a plant or an animal.

26. A method of producing a plant with improved chilling resistance which comprises:

(a) transforming a plant cell with the isolated nucleic acid of any one of Claims 1-3; and

(b) regenerating said plant with improved chilling resistance from said transformed plant cell.

27. A method of producing a plant with improved chilling resistance which comprises:

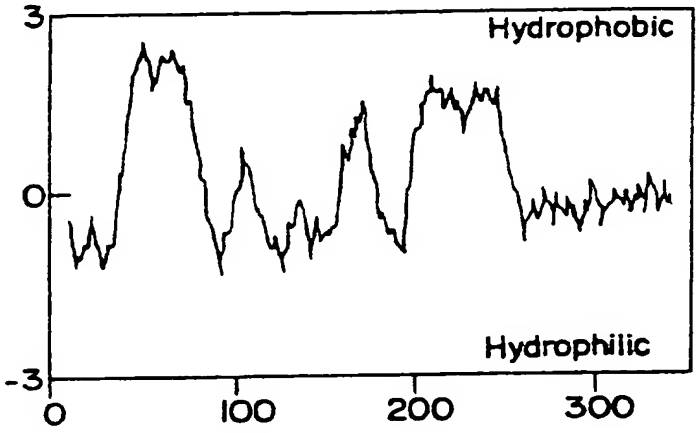

3
Hydrophobic
0
Hydrophilic
-3
0
100
200
300

FIG. 1A

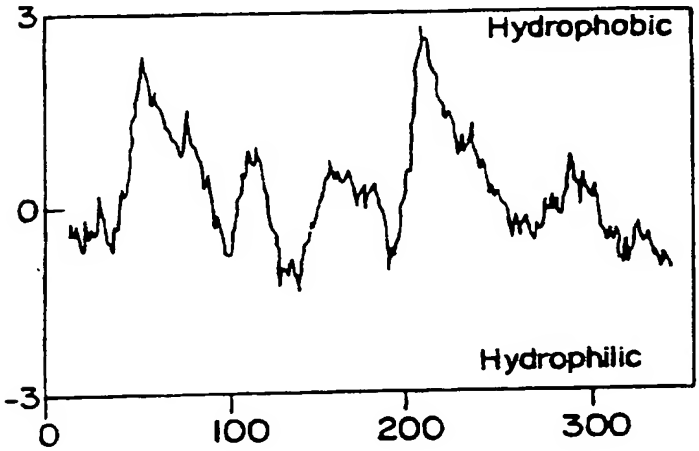

3
Hydrophobic
0
Hydrophilic
-3
0
100
200
300

FIG. 1B

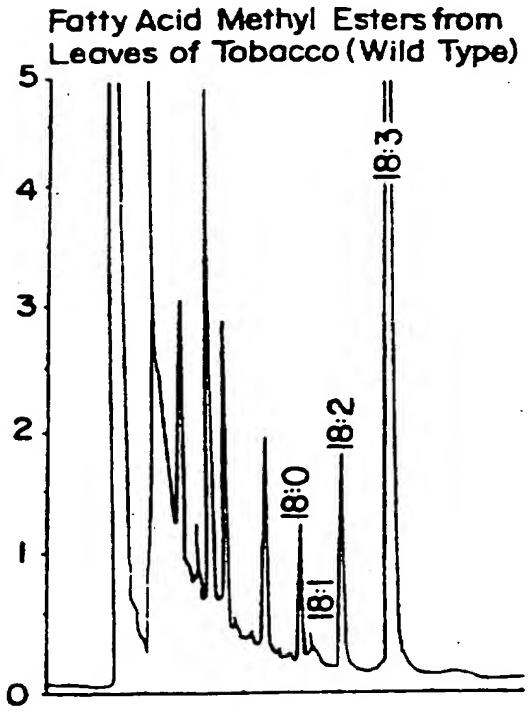

Fatty Acid Methyl Esters from
Leaves of Tobacco (Wild Type)
5
4
3
2
1
0
18:0
18:1
18:2
18:3

FIG. 4A

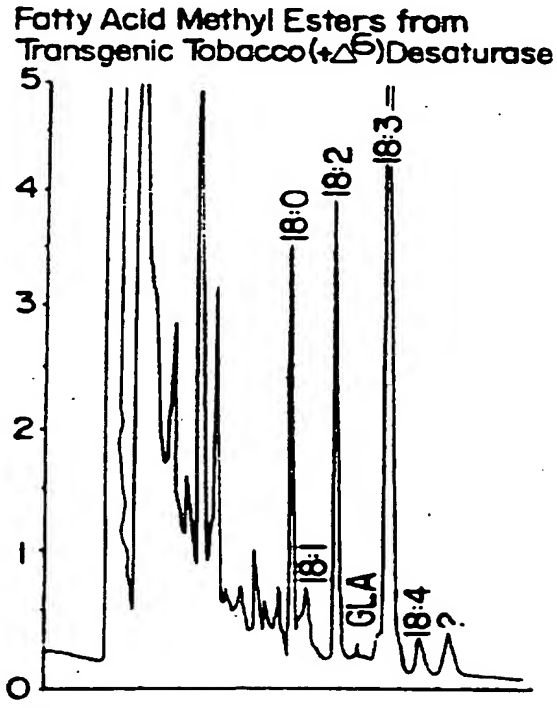

Fatty Acid Methyl Esters from
Transgenic Tobacco (+Δ6) Desaturase
5
4
3
2
1
0
18:0
18:1
18:2
GLA
18:3
18:4
?

FIG. 4B

FIG. 6

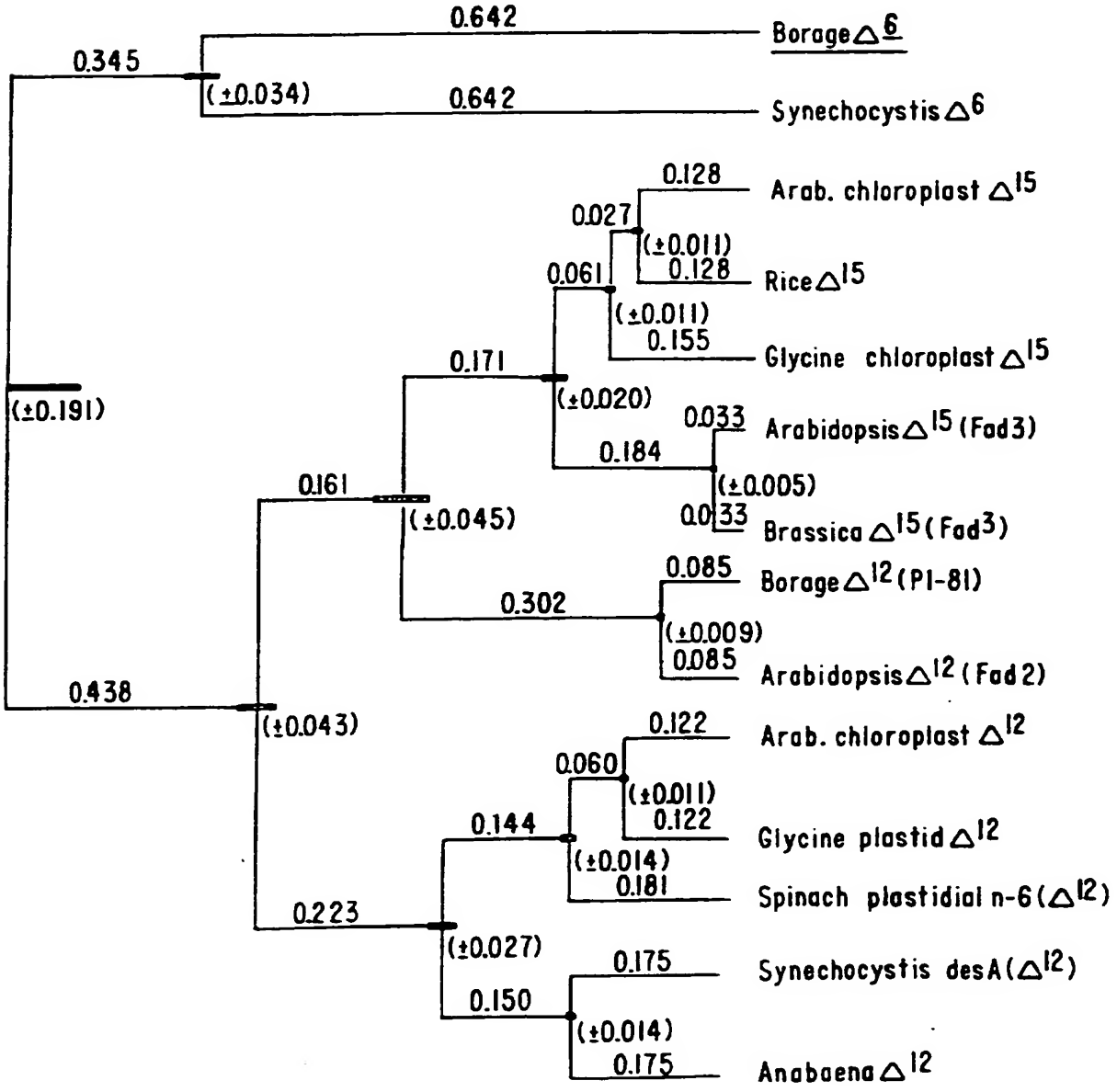

0.642
Borage Δ6
0.345
(±0.034)
0.642
Synechocystis Δ6
0.128
Arab. chloroplast Δ15
0.027
(±0.011)
0.128
Rice Δ15
0.061
(±0.011)
0.155
Glycine chloroplast Δ15
0.171
(±0.020)
0.033
Arabidopsis Δ15 (Fad3)
0.184
(±0.005)
0.033
Brassica Δ15 (Fad3)
(±0.191)
0.161
(±0.045)
0.085
Borage Δ12 (PI-81)
0.302
(±0.009)
0.085
Arabidopsis Δ12 (Fad2)
0.438
(±0.043)
0.122
Arab. chloroplast Δ12
0.060
(±0.011)
0.122
Glycine plastid Δ12
0.144
(±0.014)
0.181
Spinach plastidial n-6 (Δ12)
0.223
(±0.027)
0.175
Synechocystis desA (Δ12)
0.150
(±0.014)
0.175
Anabaena Δ12

FIG. 8A

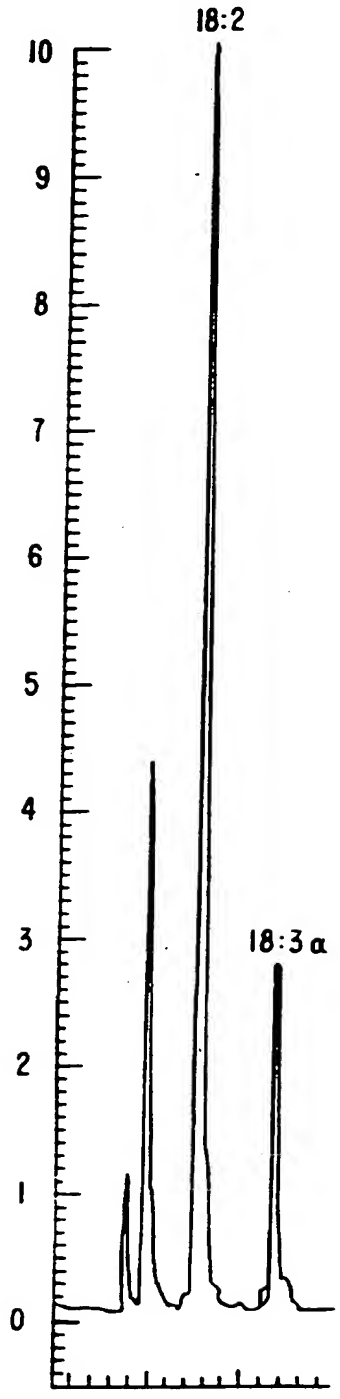


FIG. 8B

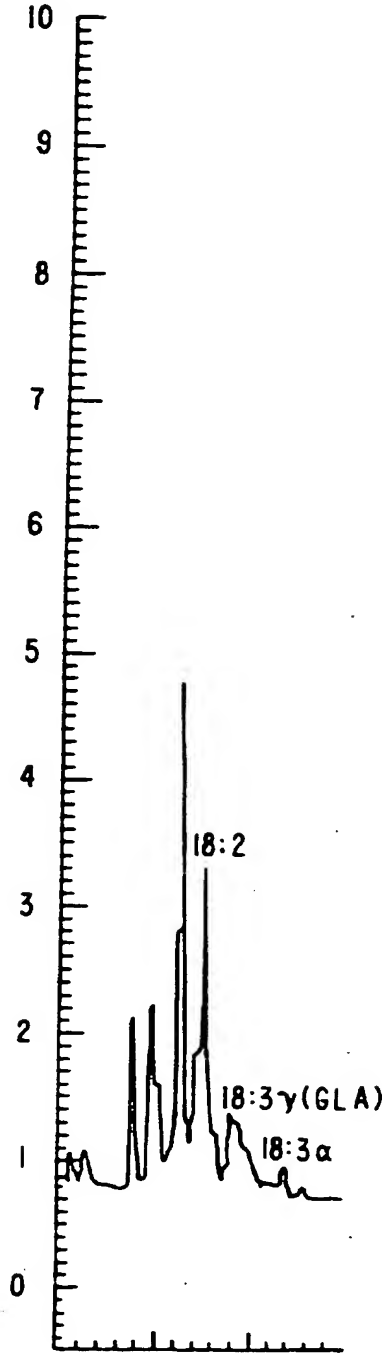


FIG. 10A

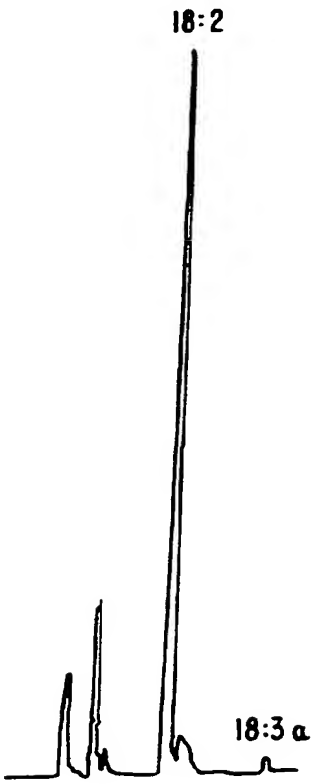


FIG. 10B